AlgAranha

FluoSpec Arduino Vs1.0

AlgAranha Team USP-UNIFESP BRASIL

Motivation

In this iGEM edition happens the third Interlab Study. On this study, teams from all over the world measure GFP fluorescence on different experiments for characterization of standard biological parts, following standard protocols and calibration solutions provided by the iGEM Headquarters for controlling instrument variability and transforming relative measurements into absolute values.

However, in order to gather your data you would rely on commercial equipment for measuring fluorescence. Those machines are usually expensive and not open-source for the community, which create barriers for anyone who would enjoy doing Science, but cannot afford the high costs! For this reason, the USP-UNIFESP BRASIL team wanted to develop a Do-It-Yourself device for measuring fluorescence, based on inexpensive materials and open-source protocols, using Arduino and Matlab.

Hardware

Spectrophotometers are equipment used in many fields of science, ranging from physics to molecular biology. Our project aims to build an OpenHardware spectrophotometer, designed to be precise, easy to modify and extremely cheap. Our team believes that the standardization of a simple and specific device could facilitate the reproducibility of analyses, since it would be possible for other teams to build the same equipment with high fidelity and low cost by following the same steps and using specifications provided.

For the construction of this equipment, we followed the idea proposed in 2014 by the Aachen team **[http://2014.igem.org/Team:Aachen]**. We started by creating a support for cuvettes in a 3D printer which allowed the best fit of the components shown in Figure 1. Next, we have designed our system to use LEDs instead of a refraction optical system (Figure 1). This choice was made because the wavelength needed to promote excitation of GFP (490nm) is close to the wavelength emitted by fluorescence of the same (510nm). Therefore, placing the sensor on an angle of 90 degrees with the LED helps to reduce interference. Furthermore, as

the LED plug and filter are simple, it is possible to easily exchange them for other applications. Thus, we have prototyped our equipment as seen in Figure 2 (3D model).

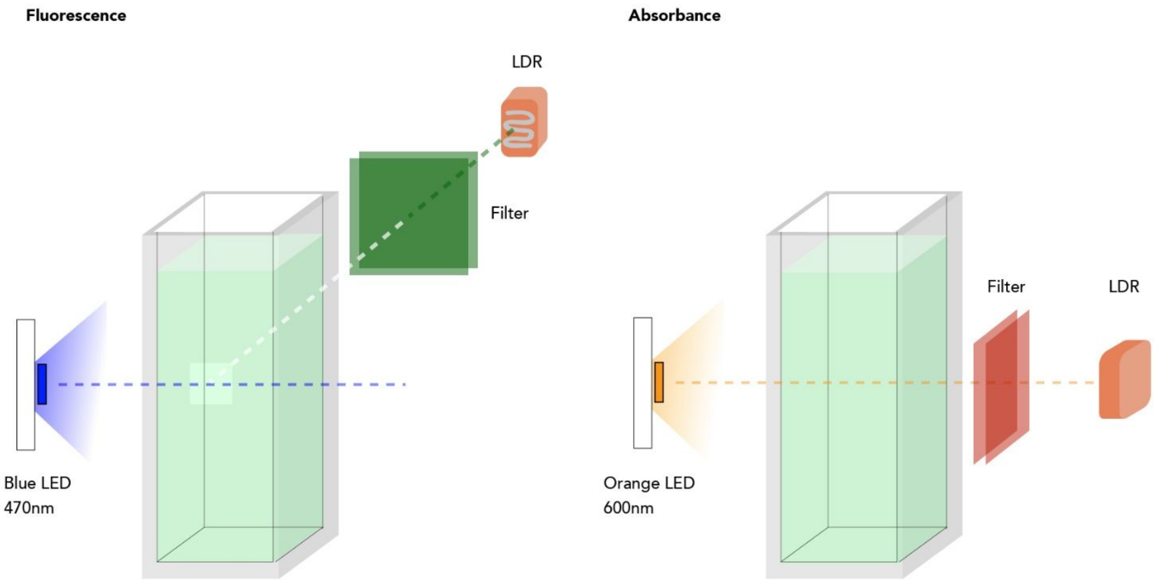

Figure 1 : On the right the demonstration of a fluorescence reader that captures the light emitted by the sample and on the left a spectrometer that captures the light that can pass through the sample.

.

Figure 2: Support 3D model displayed in all directions.

Software

Due to better data processing and handling capacity, we have chosen to develop our software *FluoSpec* (Figure 3) on Matlab, which is widely used in engineering and has additional features that allow the communication with Arduino.

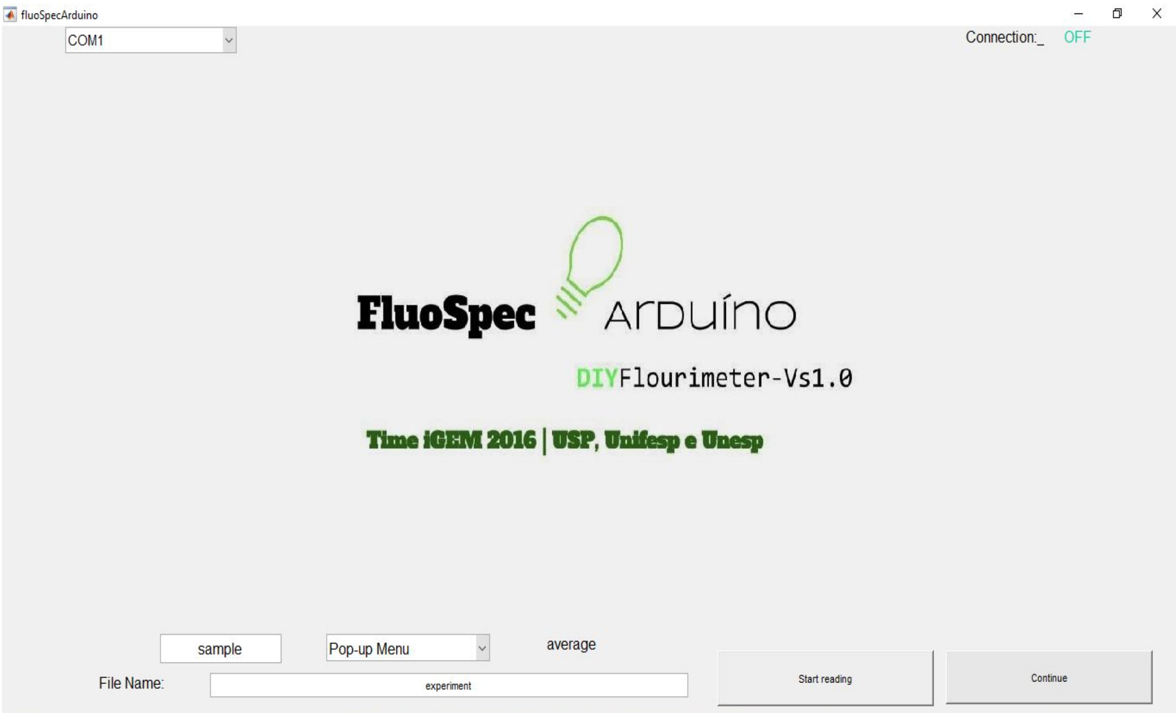

Figure 3: Home screen software developed using GUI in MATLAB.

AlgAranha

Estimated Cost

Components	cost [Real]	cost approximate[Dollar]
1 Arduino UNO R3	59,90	18,65
jumper male-female	16,90	5,26
jumper male-male	16,90	5,26
1 small breadboard	16,90	5,26
1 LDR	1,40	0,44
1 LED 480 nm	0,15	0,05
Total	112,15	34,92
considering the dollar to R$	3,2114	

Figure 4: Table containing the components used in this project and the respective hyperlinks to their distributors. The products were purchased in Brazil, however, as most of them are imported from the USA we converted the values by dividing the original price (in the Brazilian currency) by the dollar value on 11.10.2016

How to use

1. **Build the device:**

With the printed support and the other components in hand, we can set the final device, we represent the construction of the Arduino electric circuit using the software Fritzing as shown in Figure 5 and Figure 6.

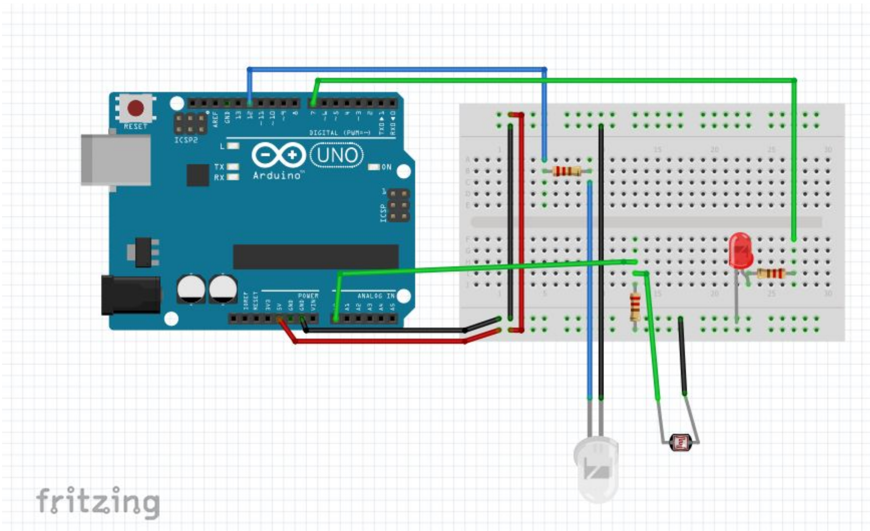

Figure 5: Circuit representation

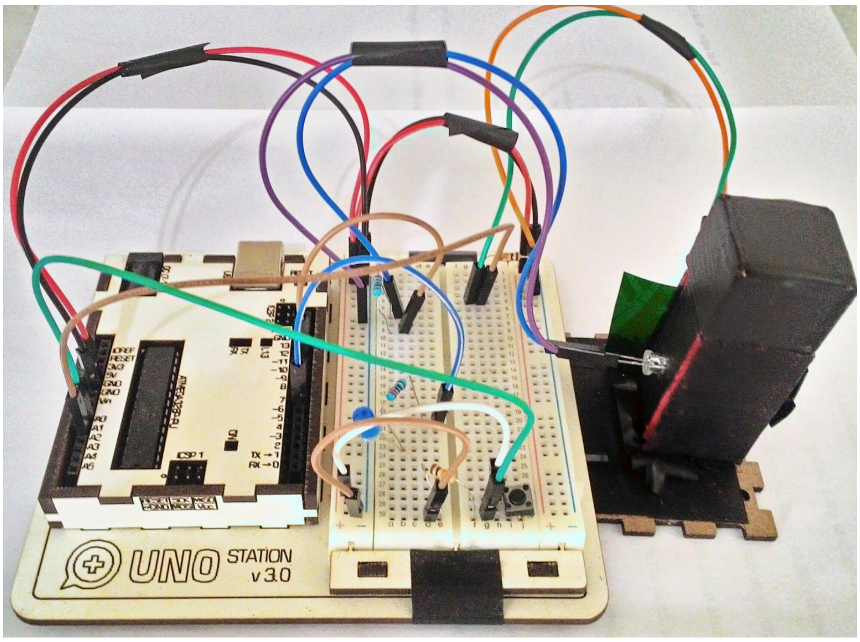

Figure 6: Photo of the developed prototype.

2. **Install the software:**
    1. Arduino
    2. Matlab

3. **Step by step:**
    1. Download the **Matlab support for the Arduino**: You need to run *install_arduino.m* file in matlab and *adioes.pde* file in arduino so matlab can communicate with the device.

2. Then open the *FluoSpecArduino.m* file in matlab. When you run the file, a GUI will open (**Figure 7**), in it you must choose which port the Arduino is using for communication, the filename that you want to save your data and then click in the *Start Reading* button, when you click in this button, you will see the message *Attempting connection* (**Figure 8**).
3. After reading,a graph is automatically plotted, showing the 10 readings for that sample and its average **(Figure 9)** if you are making the second reading, after 2 seconds, the chart will change to show the readings over time (**Figure 10**).
4. You must be careful because the parameter that determines where the readings will be saved is the popup menu, if you select a name to a sample, remember to always select it from the menu, otherwise a new sample will be created with the same name. On this screen, you have the option to continue reading (by clicking in Start Reading) or end (by clicking the End button). The End button stops the connection with Arduino and saves the file in Excel (**Figure 10**). It is important that you do not do another reading in a file that already exists before clicking the Continue button (**Figure 7**).

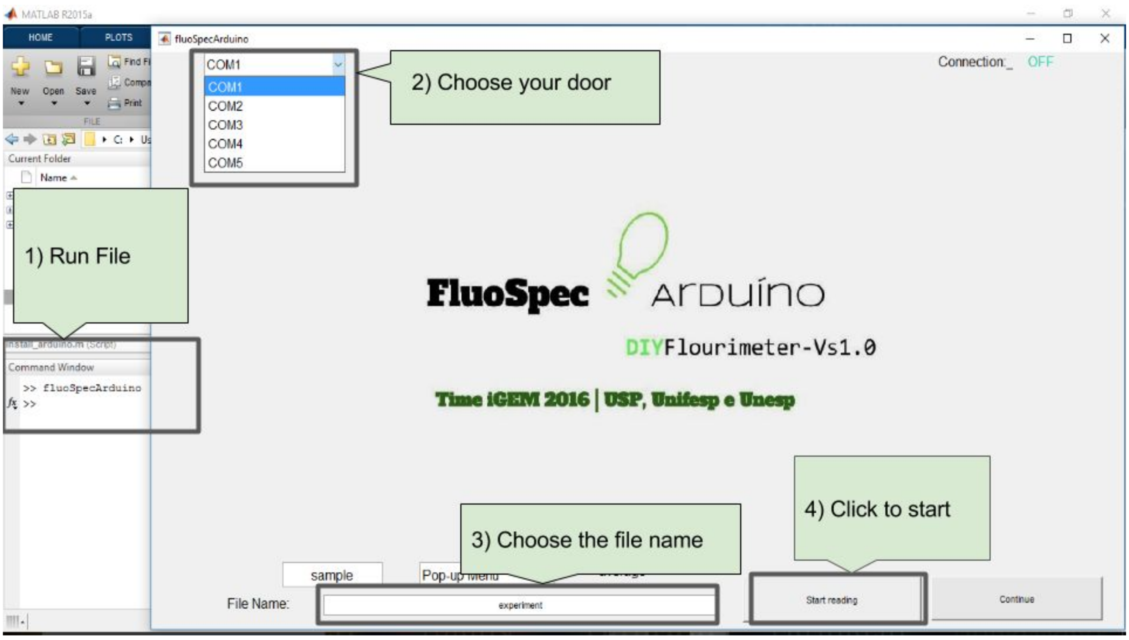

Figure 7: Home screen software showing the four steps to start reading.

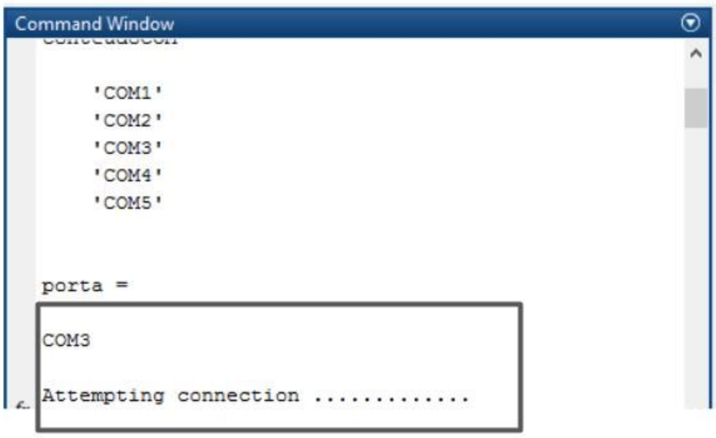

Figure 8: Matlab Command Window showing the communication message with the Arduino.

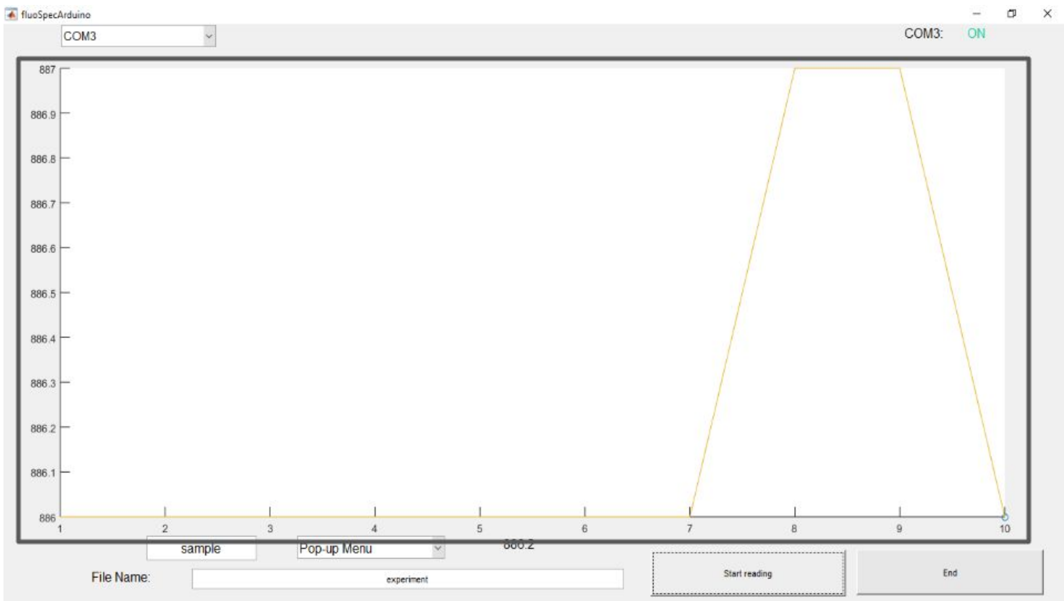

Figure 9: Graph presented after the first reading performed by the software.

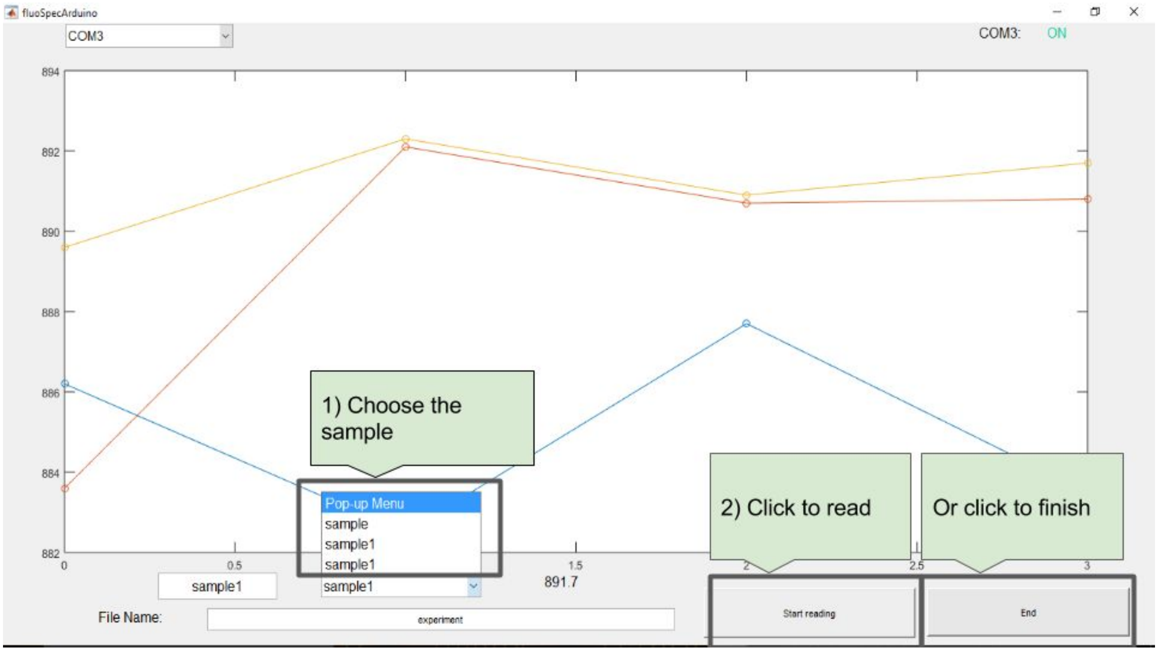

Figure 10: At the bottom the graph displayed after consecutive readings of samples in the software. The boxes show the step by step to choose the sample and continue the reading or end it.

Results

We were able to show by the FITC calibration curve (define for **interlab** study) that a linear regression of FluoSpec Arduino seems to work as well as the plate reader or fluorimeter. Therefore, our device can be used to determine fluorescence, but still need to test in a biological sample for a more precise operation of its operation. **(Figure 11)**

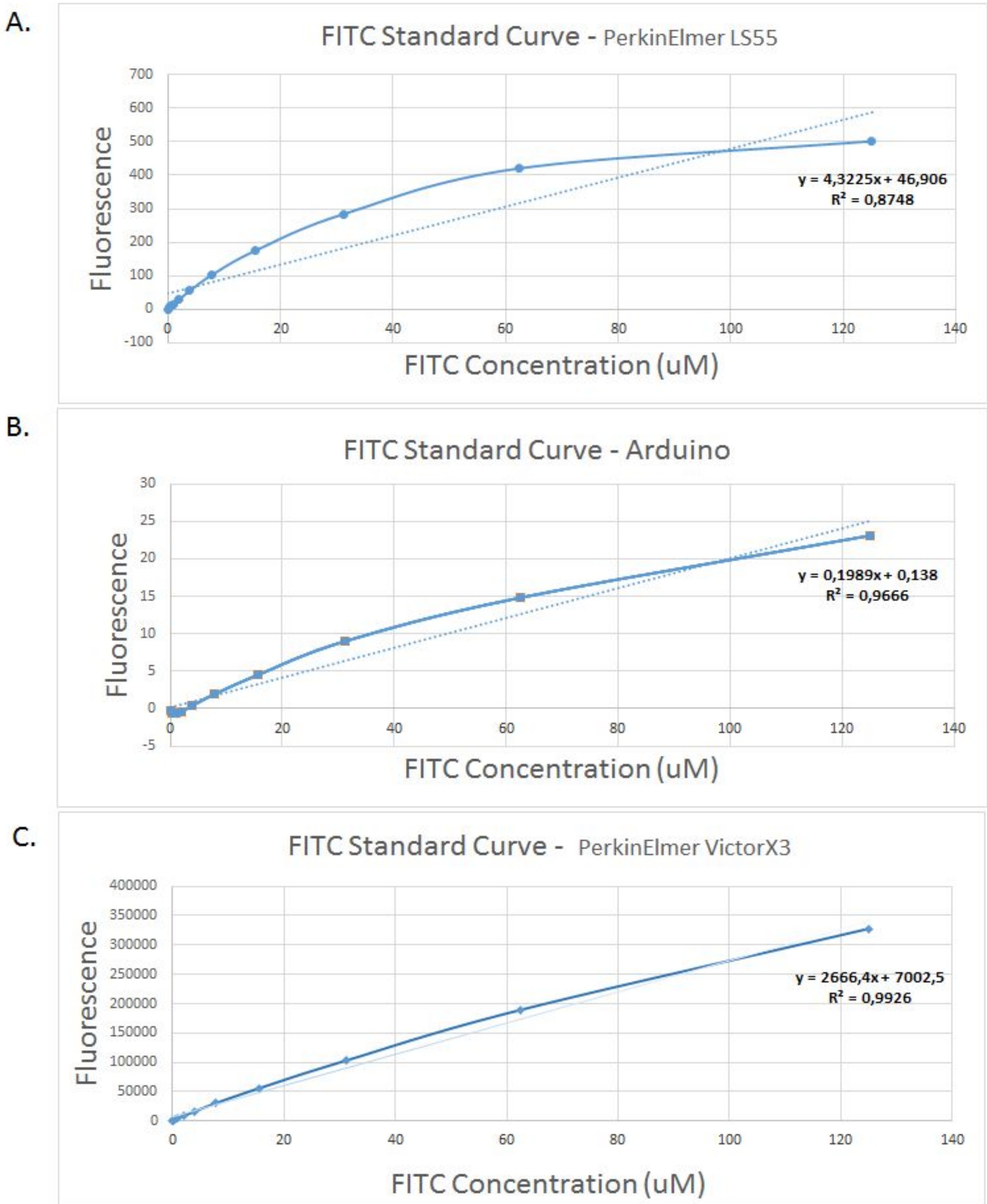

Figure 11: Comparison of the calibration curve with FITC in: (A) Fluorimeter, (B) FlouSpec Arduino and (C) Plate reader.

AlgAranha

Devenlopers

- **João Vitor Dutra Molino:** Universidade de São Paulo Faculdade de Ciências Farmacêuticas Av. Prof. Lineu Prestes, 580, B16 Cidade Universitária 05508000-São Paulo, SP +55 11 3091 2376 molino@usp.br
- **Victor Nunes de Jesus:** Universidade Estadual Paulista “Júlio de Mesquita Filho” Av. Dom Antonio, 2100 Bairro: Parque Universitário 19806-900 - Assis, SP Telefone: (18) 3302-5800. victor.jesus@gmail.com
- **Diogo Lima Bezerra da Silva:** Universidade Estadual Paulista “Júlio de Mesquita Filho” Av. Dom Antonio, 2100 Bairro: Parque Universitário 19806-900 - Assis, SP Telefone: (18) 3302-5800. diogolimabsilva@outlook.com

unesp

USP Universidade de São Paulo

UNIFESP Universidade Federal de São Paulo 1933